

TRANSPLANTATION OF THE UPPER REPRODUCTIVE TRACT TO THE SPLEEN IN THE FEMALE RAT: A METHOD FOR STUDYING THE PITUITARY-GONADAL RELATIONSHIP^{1, 2}

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ABSTRACT

A surgical procedure was developed for autoplasmic grafting of the ovary into the splenic circulation. The ovarian transplant is joined to the spleen by an attached piece of oviduct and uterus. Direct arterial connections to the graft are maintained throughout the procedure; there is no direct contact of splenic and ovarian tissue. Transplantation of the left utero-tubo-ovarian segment and removal of the contralateral ovary was performed best in immature rats. The ovarian artery and vein were removed from the graft one week later. The transplanted ovary hypertrophied while the uterus *in situ* remained atrophic. The relationship between the weight of the splenic ovary and the uterus *in situ* was useful in evaluating ovarian-graft response to the increased gonadotropin secretion which occurs. Gonadotropin secretion in this situation is discussed in light of possible progesterone feedback to the hypothalamo-hypophyseal system.

INTRODUCTION

Autoplasmic intrasplenic grafting of one ovary accompanied by removal of the contralateral gonad has been a convenient method of studying the ovarian-pituitary relationship in the rat (Desclin *et al.*, 1962), mouse (Leavitt and Wright, 1965), rabbit (Koulischer, 1960), and guinea pig (Lipschutz *et al.*, 1946). That the grafts secrete active steroids locally is well demonstrated by growth of uterine co-transplants; however, the uterus *in situ* remains atrophic (Gitsch, 1958). Because the liver can inactivate androgen (Biskind, 1940; 1941), estrogen (Biskind and Biskind, 1942), and progesterone (Engel, 1946), it is implied that steroids secreted by the splenic ovary are metabolized to inactive forms by the liver. Gonadotropin hypersecretion occurs (Miller and Pfeiffer, 1950), ovarian hypertrophy ensues, and neoplasms can develop after about six months (Biskind and Biskind, 1944; 1949).

Although intrasplenic ovarian preparations are functional, it has not been established that ovarian tissue in the graft is entirely normal. This may be especially true during early stages of development when no direct vascular connections exist between the gonad and adjacent splenic tissue (Biskind and Biskind, 1949). Even after vascular channels have formed, there is the problem of evaluating ovarian morphology when the majority of the embedded graft is hidden by splenic tissue.

The present study was undertaken in an attempt to circumvent these difficulties. A successful procedure for autotransplanting the ovary to splenic circulation was devised in which the ovary is connected to the spleen via an attached piece of oviduct and uterus. The rationale was to sever the left uterine horn and to pass the cut end through a small hole punctured in the spleen. Blood from the ovarian artery should then supply the ovary, its attached oviduct, and uterine horn, while new vascular connections develop between the spleen and the graft. After splenic blood supply to the graft has been established, the original ovarian vascular connections can be removed at laparotomy, leaving the graft totally dependent

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on the spleen for vascular supply and drainage. Steroids secreted by the graft will then pass via the splenic vein into the hepatic portal vein and be inactivated by the liver before reaching the general circulation. Some aspects of ovarian development in this situation are reported here.

METHODS

General

The uterine artery and vein anastomose with the ovarian artery and vein, respectively. Blood flow should be possible in either direction, depending on the differential pressure between the uterine and ovarian blood vessels. To establish that the blood flow can be bidirectional in the ovarian-uterine vascular complex, 0.1 ml of an India ink-saline suspension was injected into the ovarian artery of a diestrous rat, and its course followed caudad into the uterine artery. Conversely, ink injected into the uterine artery of a similar rat was seen to pass cephalad into the ovarian artery.

In a preliminary study involving 32 adult female Sprague-Dawley rats (Maxfield Animal Supply, Cincinnati, Ohio), various problems associated with the surgical procedure were investigated. Ether proved to be the best anesthetic. There was poor recovery following the use of Nembutal given intraperitoneally (IP) at 40 mg per kilo. A left lateroventral incision about three cm long was sufficient to expose the left side of the genital tract and the caudal tip of the spleen. Incisions made near the ventral midline were usually associated with a high incidence of adhesions between the incision and graft.

Transplantation Technique

The procedure finally adopted is depicted in figure 1 and involved the following:

1. The left uterine horn was ligated near the middle of its length, care being taken to include the uterine artery and vein within the ligature.
2. A hole just large enough to accommodate the uterus was punctured in the caudal pole of the spleen with a sharp 13-gauge trocar. The trocar was left in the puncture until the uterine horn had been cut free.
3. The left uterine horn was severed above the ligature, and its free end then quickly passed through the splenic puncture. The uterus was pulled through the puncture until the ovary was flush with the spleen on one side, and the graft was anchored in place by passing a short 000-gut suture through the uterus on the other side of the spleen.
4. The graft was placed under the caecum, the muscular wall closed with interrupted gut sutures, and the skin closed with nine mm wound clips.
5. The contralateral (right) ovary was then extirpated in the usual manner through a small dorsolateral incision on the right side. It was weighed fresh and preserved in Bouin's fluid for possible future histologic study. No special post-operative care was necessary, though penicillin G at 2000-5000 units was routinely administered IP at closure.

Laparotomy

Because vascularization between the spleen and embedded uterus was well established within one week, the ovarian artery and vein were cauterized during laparotomy at this time. Often the ovarian capsule was distended with fluid, and it was found advisable to open the capsule. The ovary was easy to observe at this time, and photographs of the graft were taken routinely.

Experiment 1—Ovarian Development after Autotransplantation to the Spleen in Hemiovariectomized Immature Females

Luteal and follicular development of ovaries grafted to the spleen in adult animals was somewhat variable, probably because transplantation was carried out

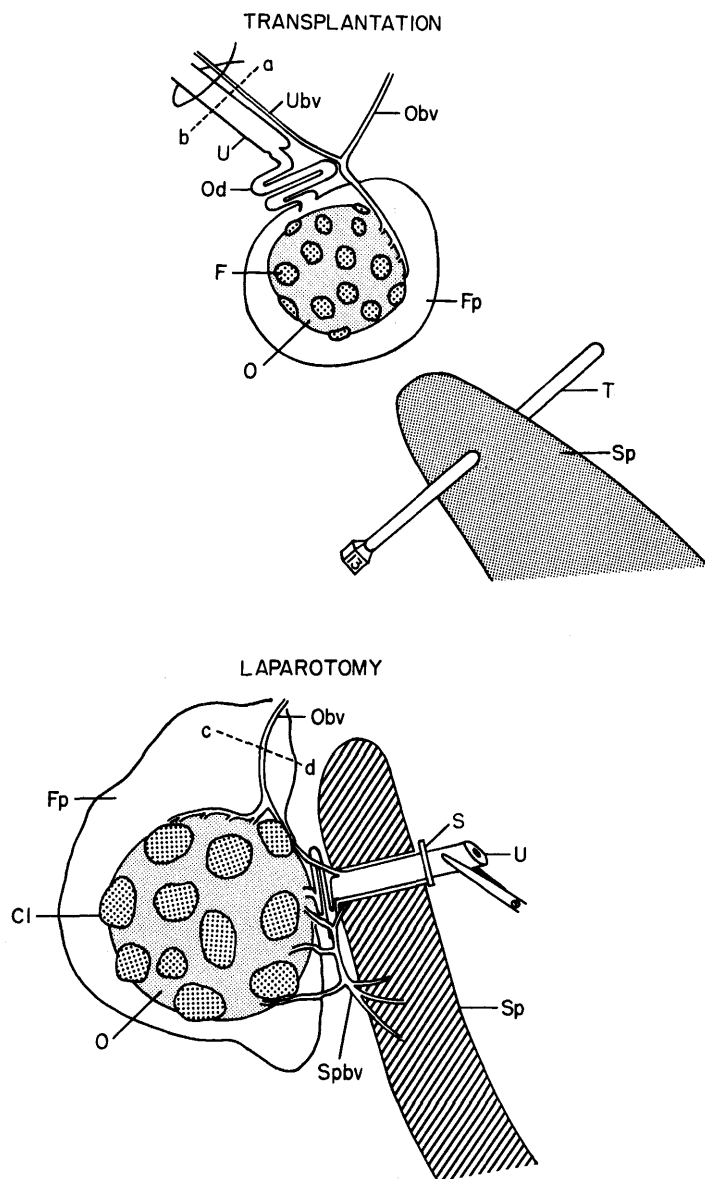


FIGURE 1. Diagrammatic representation of the surgical procedure. Transplantation is at week 1, laparotomy at week 2.

a—b	plane of uterine transection	Od	oviduct
c—d	plane of cautery	S	anchoring suture
Cl	corpora lutea	Sp	spleen
F	follicles	Spbv	splenic blood supply to the graft
Fp	fatpad	T	trocar
O	ovary	U	uterus
Obv	ovarian blood vessels	Ubv	uterine blood vessels

without regard to the stage of the estrous cycle. Hence, it was considered advantageous to use immature animals in which all ovaries are at a comparable stage of development.

Fifty female CD rats (Charles River Breeding Labs., Wilmington, Mass.) were subjected to the transplantation operation at 30 days of age. The ovarian artery and vein were cauterized one week later (37 days of age). The grafts were allowed to develop 53 days after laparotomy (90 days of age), at which time the animals were sacrificed by cervical dislocation.

Daily vaginal smears were taken by lavage, and periods of vaginal cornification after laparotomy indicated the presence of vascular adhesions between the graft and peripheral circulation.

Experiment 2—Early Development of the Ovarian Graft

Little information was available on the growth of splenic ovaries. Therefore, some of the early changes accompanying transplantation of the utero-tubo-ovarian segment in hemiovariectomized rats were studied.

From 48 CD females, 30 were placed in Group I and subjected to the transplantation operation at 30 days of age (week 1). The remaining 18 animals (Group II) were either given surgical trauma (12 animals) or left undisturbed (six animals). Surgical trauma consisted of a mock transplantation operation performed at week 1. On week 2, 25 of the rats in Group I were subjected to laparotomy and the ovarian artery and vein cauterized. Also on week 2, the remaining five animals in Group I, and three animals in Group II were sacrificed. A similar complement of animals was sacrificed at weekly intervals during the next five weeks (weeks 3-7). At sacrifice, adenophypophysis, adrenals, ovaries or ovarian graft, and untransplanted portion of the uterus were weighed. All organs were rapidly extirpated, trimmed, and rolled on filter paper prior to weighing. The uterus was split longitudinally, and, since part of the left horn had been removed at transplantation, only the right half was weighed.

RESULTS

Experiment 1—Ovarian Development after Autotransplantation to the Spleen in Hemiovariectomized Immature Females

Thirty-one of the 50 rats bearing grafts (62 per cent) had significant periods of vaginal cornification, indicating a high incidence of vascular adhesions. The presence of adhesions to the peripheral circulation which allowed the escape of active steroids was confirmed at sacrifice. Data from the 19 animals with no demonstrable adhesions suggested an increase in growth rate (body weight = 270 ± 5.3 g), and in adrenal weight (71 ± 3.6 mg). The weight of the ovarian graft (430 ± 54 mg) was significantly higher than the weight of both ovaries (74 ± 4.1 mg) from normal intact females of the same age. A typical ovarian graft 60 days after transplantation is shown in figure 2. The weight of the uterus *in situ* (72 ± 7.1 mg) was significantly lower than normal (385 ± 18 mg), as would be expected if steroids from the splenic ovary were inactivated by the liver.

Experiment 2—Early Development of the Ovarian Graft

There were no differences between animals surgically traumatized or left intact in Group II. Therefore, data from these were pooled. Only three rats in Group I had adhesions, and results from these were eliminated from the data. The lower incidence of adhesions in this experiment may be attributed to better surgical technique. It was apparent that moderate experience with the procedure is prerequisite to the preparation of successful grafts.

Table 1 shows that the operation had little effect on adenohipophyseal or adrenal weight. The uterus *in situ* in Group I animals was atrophic by week 3, one week after cautery of the ovarian artery and vein, and this condition persisted

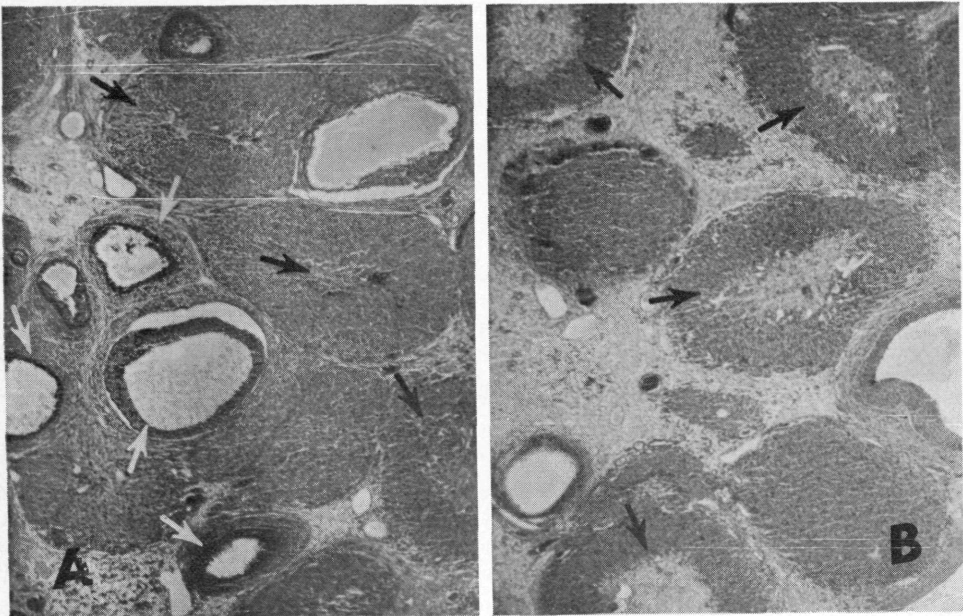


FIGURE 2. Histologic appearance of a 60-day ovarian graft weighing 752 mg. *A* shows a central region in the graft, and *B* is from the periphery. Luteal bodies (dark arrows) are found throughout, and atretic follicles (white arrows) of various sizes are shown in *A* with abundant theca surrounding them. Hematoxylin and eosin, $\times 42$.

TABLE 1

Mean organ and body weights of intact and transplant rats over a five-week period (Expt. 2)

Time* (weeks)	Organ†								Body weight	
	Adeno- hypophysis		Adrenals		Uterus‡		Ovary§		(g)	
	Grp. I	Grp. II	Grp. I	Grp. II	Grp. I	Grp. II	Grp. I	Grp. II	Grp. I	Grp. II
2	.005	.005	.025	.023	.068	.056	.033	.023	112	112
3	.006	.007	.022	.023	.031	.063	.035	.029	157	152
4	.006	.006	.025	.019	.023	.045	.068	.022	212	190
5	.006	.006	.027	.024	.016	.059	.060	.021	236	204
6	.005	.006	.026	.027	.013	.079	.071	.021	252	217
7	.005	.005	.019	.025	.012	.078	.071	.019	283	233

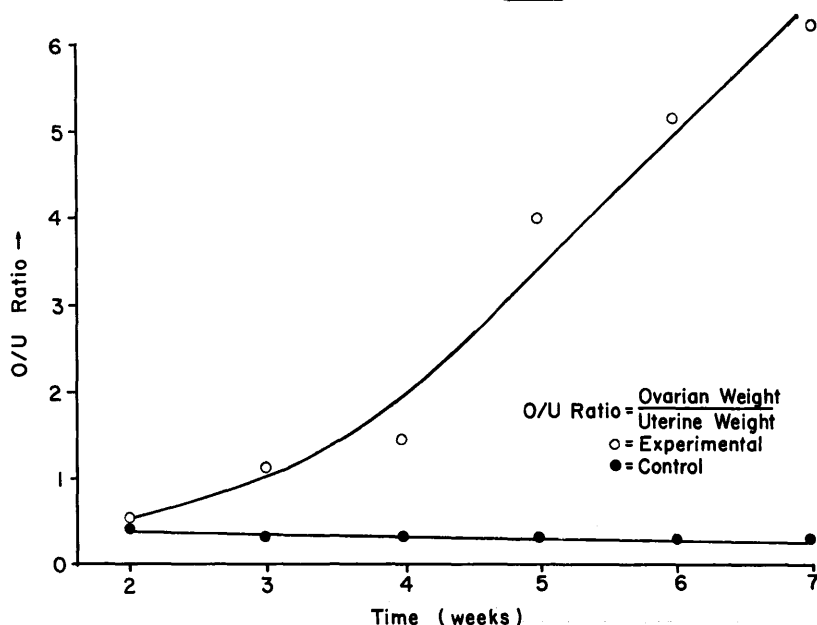
*Weeks 2 through 7 correspond to 37 through 62 days of age.
†Organ weights are expressed as per cent body weight. Five experimental animals and three controls were sacrificed each week.
‡Only the right half of the uterus was weighed.
§The weight of the experimental ovary is compared to the weight of both control ovaries.

through week 7. However, the transplanted uterine segment during the same period was fluid filled and hypertrophic. The splenic ovary increased in weight throughout, while ovaries *in situ* were maintained at a relatively constant weight. Of interest was the increased growth of hemiovariectomized rats with utero-tubo-ovarian grafts. At week 7 the body weight of animals in Group I was significantly higher than that of animals in Group II ($P < .05$).

The relationship between the uterus and the ovary *in situ* is rather constant when the ovary is in its normal location. This relationship is clearly different when the ovary has been connected to the splenic circulation. It was felt that a weight ratio of these two organs might be of value in expressing this difference.

The relationship between the ovary (O) and the uterus *in situ* (U) is plotted graphically in figure 3. In controls, the O/U ratio was constant. This illustrates

Fig. 3. Relationship of the ovary and uterus in situ in normal and transplant rats



the balanced hypophyseal-ovarian relationship in the normal female. However, the O/U ratio in experimentals increased immediately after laparotomy on week 2, and rose progressively in subsequent weeks. Although more information is needed, it seems evident that ovarian growth reflects elevated secretion of hypophyseal hormones. Representative ovarian growth sections from each week of the study are presented in figures 4-9. As the ovarian graft hypertrophied, increasing amounts of luteinized LH was seen to develop. This indicates that hypersecretion of pituitary LH as well as of FSH is occurring.

DISCUSSION

The method presented here for grafting the ovary into the hepatic portal drainage has several advantages over older techniques. Of prime importance is the fact that blood supply is not interrupted during autoplasmic transplantation. Gross ovarian morphology can be observed at laparotomy at any time during the

period of study. Hypertrophy of the ovarian graft is not impeded by surrounding splenic tissue, and there is less likelihood of tissue interaction between the spleen and ovary.

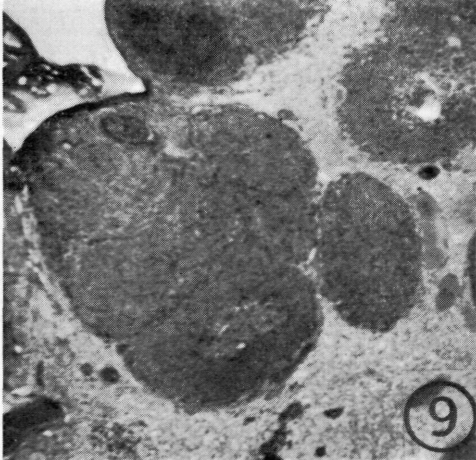
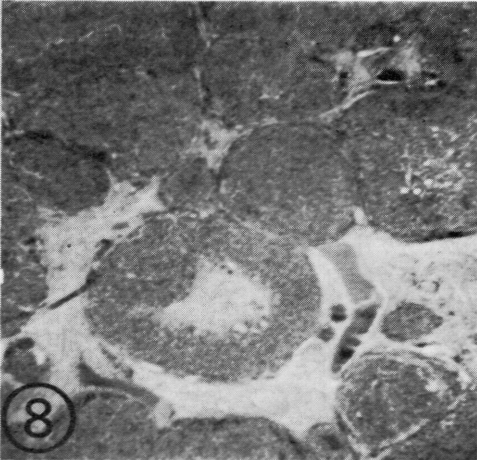
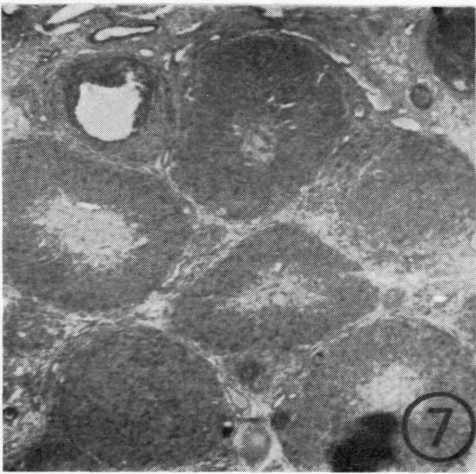
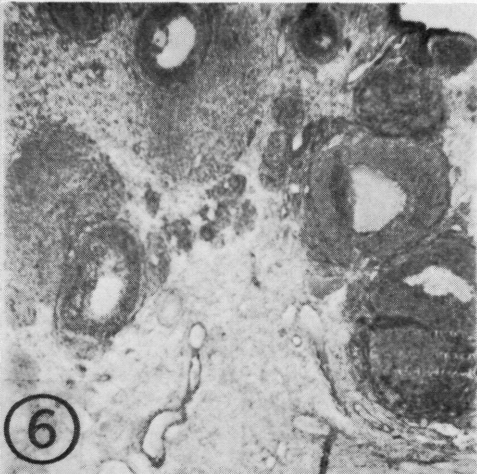
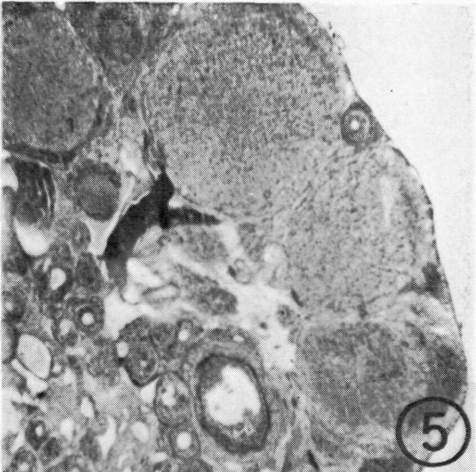
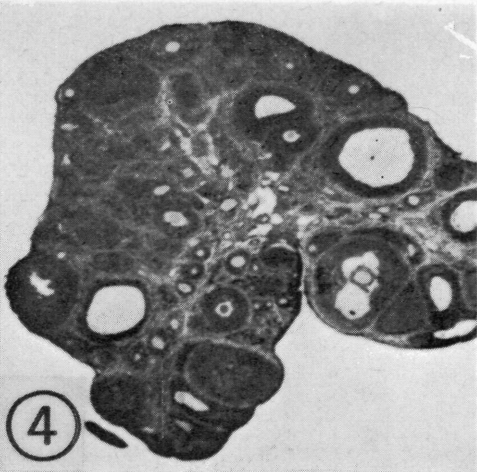
That hypertrophy of splenic ovaries is due to hypophyseal gonadotropin hypersecretion is well documented (Ely, 1959; Inoue, 1961, 1962; Miller and Pfeiffer, 1950). However, the actual mechanism controlling elevated adeno-hypophyseal hormone secretion in this situation has not been clearly established. Early stimulation of the ovarian graft from the time of transplantation until ovarian blood vessels are removed may be attributed to compensatory ovarian hypertrophy, which normally follows hemicastration in the female. Edgren *et al.* (1965) felt that this was due to increased gonadotropin secretion. Because they could not substantiate this interpretation with available assay methods for LH and FSH, confirmation of this explanation remains to be made.

Gonadotropin secretion, after interruption of ovarian blood vessels, was increased over previous levels within one week, as demonstrated by an increase in ovarian graft weight and in the O/U ratio. Associated with the progressive weight increase of ovarian grafts is the development of both stromal and parenchymal elements. Follicular development occurs, but most follicles show early luteinization and, in older grafts, conversion into clumps of luteal bodies. These changes are attributed to the hypersecretion of adeno-hypophyseal LH and FSH. It has been commonly assumed that inactivation, by the liver, of estrogen secreted from the graft triggers a post-castration type of gonadotropin secretion (Biskind and Biskind, 1944; Desclin *et al.*, 1962; Golden and Severinghaus, 1938). Greep and Jones (1950) state that, in these rats, there is a castrate type of gonadotropin secretion because of estrogen withdrawal. Achilles and Sturgis (1951) and Kullander (1956a) agree that hepatic inactivation of estrogen and androgen occurs, but report that gonadotropin secretion, while high, is not of the castrate type. Kullander (1956b) found that enough progesterone passed through the liver unaltered to produce peripheral effects on the uterus. The feedback of progesterone to the hypothalamo-hypophyseal system is known to influence LH secretion by the adeno-hypophysis (Barracrough *et al.*, 1964). Also, the possibility that estrogen inactivation products liberated by the liver might alter gonadotropin secretion should be considered (Smith, 1944).

The increased growth rate of animals with splenic ovarian grafts was also noted by Kullander (1956a). This effect may be similar to elevated growth following castration (Holt *et al.*, 1936), but the possibility remains that increased steroid secretion by the ovarian graft may stimulate liver metabolism, leading to

EXPLANATION OF FIGURES

- FIGURE 4. Section of a 9-mg right ovary from a 30-day rat (week 1), removed during transplantation. The contralateral ovary was grafted to the spleen. Note the typical immature condition characterized by numerous follicles in various stages of development. H & E, $\times 26$.
- FIGURE 5. A 38-mg ovarian graft at laparotomy (week 2). Four normal *corpora lutea* are shown indicating that ovulation has occurred during compensatory hypertrophy of the graft. Many developing follicles are present. H & E, $\times 26$.
- FIGURE 6. A 67-mg ovarian graft one week post-laparotomy (week 3). The stroma has enlarged, and luteal bodies are shown which apparently develop from proliferating thecal elements. H & E, $\times 26$.
- FIGURE 7. The periphery of a 152-mg ovarian graft three weeks post-laparotomy (week 5). A cluster of six luteal bodies is shown, in some of which a central fibrous core is present. H & E, $\times 26$.
- FIGURE 8. Several closely packed luteal bodies are shown near the margin of a 321-mg ovarian graft at week 6, four weeks post-laparotomy. H & E, $\times 26$.
- FIGURE 9. A conglomerate of several luteal bodies surrounded by abundant stroma in a 323-mg ovarian graft at week 7. Not shown are the scattered follicles which are still present at this time. H & E, $\times 26$.



increased growth. It would seem important to identify steroids secreted from splenic ovarian grafts, both those that pass unaltered through the liver and those that are conversion products liberated from the liver. The transplantation procedure presented here provides a system that makes *in vivo* or *in vitro* studies on this point feasible.

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LITERATURE CITED

- Achilles, W. E., and S. A. Sturgis.** 1951. The effect of the intrasplenic ovarian graft on pituitary gonadotropins. *Endocrinol.* 49: 720-731.
- Barracrough, C. A., S. Yrarrazaval, and R. Hatton.** 1964. A possible hypothalamic site of action of progesterone in the facilitation of ovulation in the rat. *Endocrinol.* 75: 838-845.
- Biskind, G. R.** 1940. Inactivation of methyl testosterone in male rats. *Proc. Soc. Exp. Biol. & Med.* 43: 259-261.
- . 1941. Inactivation of testosterone propionate by normal female rats. *Proc. Soc. Exp. Biol. & Med.* 46: 452-453.
- , and **D. E. Biskind.** 1949. Experimental ovarian tumors in rats. *Amer. J. Clin. Path.* 19: 501-521.
- Biskind, M. S., and G. R. Biskind.** 1942. Effect of vitamin B complex deficiency on inactivation of estrone in the liver. *Endocrinol.* 31: 109-114.
- . 1944. Development of tumors in the rat ovary after transplantation into the spleen. *Proc. Soc. Exp. Biol. & Med.* 55: 176-179.
- Desclin, L., J. Flament-Durand, and W. Gepts.** 1962. Transplantation of the ovary to the spleen in rats with persistent estrus resulting from hypothalamic lesions. *Endocrinol.* 70: 429-436.
- Edgren, R. A., A. F. Parlow, D. L. Peterson, and R. C. Jones.** 1965. On the mechanism of ovarian hypertrophy following hemicastration in rats. *Endocrinol.* 76: 97-102.
- Ely, C. A.** 1959. Inhibition of tumor formation in ovarian splenic implants after gonadotrophic antiserum. *Cancer Res.* 19: 37-46.
- Engel, P.** 1946. A study of progesterone inactivation by the liver after intraportal administration. *Endocrinol.* 38: 215-217.
- Gitsch, E.** 1958. Local effects of ovary on uterus after combined intrasplenic autotransplantation in castrated rats. *Endocrinol.* 62: 391-399.
- Golden, J. B., and E. L. Severinghaus.** 1938. Inactivation of estrogenic hormone of the ovary by the liver. *Proc. Soc. Exp. Biol. & Med.* 39: 361-362.
- Greep, R. O., and I. C. Jones.** 1950. Steroid control of pituitary function. *Rec. Prog. Hormone Res.* 5: 197-261.
- Holt, H., R. W. Keeton, and B. Vennesland.** 1936. The effect of gonadectomy on body structure and body weight in albino rats. *Am. J. Physiol.* 114: 515-525.
- Inoue, S.** 1961. Acyclic secretion of gonadotrophins by hypophysis in gonadectomized male and female rats. *J. Fac. Sci. Univ. Tokyo (Sec. IV)* 9: 309-318.
- . 1962. Mode of secretion of gonadotrophic hormones in gonadectomized rats as studied by parabiosis and intrasplenic ovarian transplantation. *Endocrinol. Jap.* 9: 234-235.
- Koulischer, L.** 1960. Greffes intraspléniques d'ovaire chez la lapine castrée. *Ann. Endocrinol.* 21: 314-320.
- Kullander, S.** 1956a. Studies in spayed rats with ovarian tissue autotransplanted into the spleen. II. Development from the age of 2 to 6 months. *Acta Endocrinol.* 21: 185-203.
- . 1956b. Experimental decidualoma in spayed rats with ovarian tissue autografted into the spleen. *Acta Endocrinol.* 21: 204-210.
- Leavitt, W. W., and P. A. Wright.** 1965. The plant estrogen, coumestrol, as an agent affecting hypophysial gonadotropic function. *J. Exp. Zool.* 160: 319-328.
- Lipschultz, A., H. Ponce de Leon, E. Woywood, and O. Gay.** 1946. Intrasplenic ovarian grafts in the guinea pig and the problem of neoplastic reactions of the graft. *Rev. Canadienne Biol.* 5: 181-198.
- Miller, O. J., and C. A. Pfeiffer.** 1950. Demonstration of increased gonadotrophic hormone production in castrated mice with intrasplenic ovarian grafts. *Proc. Soc. Exp. Biol. & Med.* 75: 178-181.
- Smith, O. W.** 1944. The pituitary responses of mature male rats to an oxidative inactivation product of estrone. *Endocrinol.* 35: 146-157.